

CLAIMS

1. A method of determining the suitability of a sample of mammalian semen for cooling and/or cryopreservation or storage, said method comprising:
 - (a) providing said sample of semen;
 - (b) determining the level of a hydrophobic stimulator of 11β -HSD activity in said sample; and
 - (c) assessing, from the level of 11β -HSD stimulator determined, the suitability of the semen sample for cooling and/or cryopreservation or storage.
2. A method according to claim 1 wherein said sample of semen is from a human male.
3. A method according to claim 1 wherein said sample of semen is of rodent, bovine, equine, porcine or ovine origin.
4. A method according to any one of the preceding claims wherein said hydrophobic stimulator of 11β -HSD activity elutes in a fraction from a C18 column at either 50 to 75% or 95 to 100% methanol.
5. A method according to any one of the preceding claims wherein said determination of the level of hydrophobic stimulator of 11β -HSD activity is by contacting said sample of semen with 11β -HSD present in another body fluid or another body derived substance, and determining the effect of the hydrophobic stimulator on the activity of 11β -HSD.
6. A method according to claim 5 wherein said contacting is performed by adding 11β -HSD and a substrate of 11β -HSD to said semen sample.
7. A method according to claim 6 wherein said substrate is ^3H -cortisol or ^3H -corticosterone.

8. A method according to any one of the preceding claims wherein said other body derived substance is a homogenised animal organ.
9. A method according to claim 8 wherein said animal organ is an animal kidney.
10. A method according to claim 9 wherein said animal organ is a rodent organ.
11. A method according to claim 10 wherein said rodent organ is a rat organ.
12. A method according to claim 11 wherein said rat organ is a rat kidney.
13. A method according to any one of the preceding claims wherein a control assay is conducted to allow for any 11 β -HSD already present in said sample from said male individual.
14. A method of improving the survival rate of sperm or promoting the viability of sperm, said method comprising:
 - (a) providing a sample of semen; and
 - (b) combining said sample of semen with an increased concentration of a hydrophobic stimulator of 11 β -HSD activity; and optionally
 - (c) storing said combination of semen and hydrophobic stimulator for a period of time.
15. A method according to any one of the preceding claims wherein said hydrophobic stimulator of 11 β -HSD activity elutes in a fraction from a C18 column at either 50 to 75% or 95 to 100% methanol.
16. A method according to claim 14 or 15 wherein said increased concentration of hydrophobic stimulator of 11 β -HSD activity is an amount or concentration of 11 β -HSD stimulator which, when assessed at a dilution of

10% by volume, could increase 11 β -HSD activity by 100% or more relative to enzyme activity measured in the absence of the stimulator.

17. A method according to any one of claims 14 to 16 further comprising
 - (a) a step of cooling said combination of semen and hydrophobic stimulator of 11 β -HSD activity to 5°C or below; and /or
 - (b) a step of freezing said combination of sperm and hydrophobic stimulator of 11 β -HSD activity.
18. A method according to any one of claims 14 to 17 wherein sperm is removed from said sample of semen and said sperm is combined with an increased concentration of a hydrophobic stimulator of 11 β -HSD activity.
19. A method according to claim 14 wherein said combination of semen and hydrophobic stimulator is stored without cooling or cryopreservation.
20. A method according to any one of claims 14 to 19 wherein 85% or more of said human sperm, 40% of said pig sperm, 50% of said horse sperm, 70% of said cow sperm, 50% said sheep sperm or 60% of said rodent sperm survive said cooling and/or cryopreservation or said storage.
21. A method of fertilizing an oocyte *in vitro* comprising contacting said oocyte with sperm obtained by a method according to any one of claims 14 to 20.
22. A method of performing an assisted conception/reproductive procedure comprising contacting an oocyte with sperm obtained by a method according to any one of claims 14 to 20 under conditions which allow fertilization of the oocyte.

23. A method according to claim 22 wherein said assisted conception/reproductive procedure is an IVF procedure comprising contacting said oocyte and said sperm *in vitro* and introducing the fertilized oocyte or zygote or embryo derived therefrom into a female such that it may develop to term.
24. A method according to claim 22 wherein an artificial insemination (AI) procedure.
25. A method according to claim 24 wherein said artificial insemination is an intra-uterine insemination (IUI) procedure.
26. A method according to claim 22 wherein said assisted conception/reproductive procedure is an intracytoplasmic sperm injection (ICSI) procedure.
27. A method of obtaining a hydrophobic product that improves the tolerance of mammalian semen to cooling and/or cryopreservation or storage, comprising the steps of:
 - (a) providing a sample of semen;
 - (c) removing the seminal plasma from the sperm; and
 - (d) fractionating the seminal plasma of (b) to enrich for said product.
28. A method according to claim 27 wherein said seminal plasma is removed from said sperm by centrifugation, Percoll centrifugation or Percoll swim-up.
29. A method according to claim 27 or 28 wherein said fractionating of said seminal plasma is on a C18-methanol affinity chromatography column, TLC, HPLC or FPLC.

30. A product obtainable by fractionation of mammalian seminal plasma and having a stimulatory effect on 11 β -HSD activity, which improves the tolerance of semen to cooling and/or cryopreservation or storage.
31. A product according to claim 30 which is obtainable by a method according to any one of claims 27 to 29.
32. Use of a product as defined in claim 30 or 31 to improve the tolerance of semen to cooling and/or cryopreservation or storage.
33. Use of a product as defined in claim 30 or 31 in the manufacture of a medicament for use in the treatment of inflammatory disease by increasing the survival of topically applied cortisol or cortisol already circulating within the bloodstream.
34. Use of a product as defined in claim 30 or 31 in the manufacture of a medicament for use in the treatment of inflammatory disease by stimulating the production of cortisol from circulating cortisone by stimulation of 11 β -HSD1.